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Alfalfa physiological response to potato leafhopper injury depends on leafhopper and alfalfa developmental stage

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Abstract

We examined the effects of potato leafhopper (*Empoasca fabae*) developmental stage and alfalfa (*Medicago sativa*) developmental stage on the physiological response of the plant to injury. We used radioactive carbon dioxide to label the photoassimilate stream and evaluate the phloem health of alfalfa. In one experiment, six first instar, four fourth instar, and three adult leafhoppers were caged by stage on single alfalfa stems for approximately one day. Only fourth instar nymphs significantly reduced the amount of label transported to injured tissues above the source of the labeled assimilate. First instar nymphs had no effect and adults reduced assimilate transport to stem tips, but this trend was not significant possibly because of confounding variables. However, injury by both first instar nymphs and adults resulted in greater concentration of labeled assimilate in portions of the stem below the feeding site. In another experiment, the developmental stage of alfalfa stems was central to the physiological response of alfalfa to leafhopper injury. A 20 h exposure to three adult leafhoppers significantly reduced the amount of label translocated to the tip and crown tissues of early vegetative plants, and to the crown tissue only of late vegetative plants. In reproductive plants, assimilate translocation was not affected by leafhopper injury. In a final experiment, we found no evidence of an effect on the photosynthesis of leaves of similar age and position to those used as source leaves in our translocation studies. Our findings contribute to our understanding of the physiological response of plants to injury by sap-feeding insects, and suggest the need for greater refinement of economic injury levels based on leafhopper and plant developmental stage.

Introduction

Translocation is a key process by which plants redistribute carbon from the site of fixation to regions of assimilate storage and use. Sap-feeding herbivores exploit this sugar and nutrient redistribution process by tapping phloem tissues (Raven, 1983). However, the quality and distribution of this carbon resource varies greatly in space and time. Perennial plants with annual herbage, like alfalfa, *Medicago sativa* L., undergo cyclic changes in the direction and magnitude of assimilate transport (Pearce et al., 1969; Ueno & Smith, 1970). Concurrently, their stems pass through developmental stages during which both the risk and consequences of sap feeding vary.

The injurious effects of shoot-feeding, photoassimilate-consuming insects, like potato leafhopper, *Empoasca fabae* (Harris), are expected to vary with plant developmental stage. Smaller alfalfa stems (about 7 cm) suffer nearly twice the yield reductions (fresh weight) as larger (20 cm) stems (Kouskolekas & Decker, 1968). Disruption, delay, or blockage of phloem translocation (Nielsen et al., 1990) impede carbon supply for energy, cell wall construction, and other growth activities such as adding and enlarging cells and, thereby, greatly impact regrowth. Removal of photoassimilate may also trigger a compensatory increase in photosynthesis. Consequently, the response of stems under attack by sap-tapping herbivores can be determined by their ability to tolerate

physical injury and compensate for lost assimilates. Furthermore, the effects of carbon removal and disrupted assimilate flow are expected to have unique effects on stems at different stages in the regrowth cycle.

Herbivore life-stage can determine the degree to which plant physiology is altered. The ability of vascular-feeding insects to disrupt nutrient movement and partitioning varies with the insect's growth and development. For alfalfa, older potato leafhopper stadia (third instar through adult stage) are more injurious to herbage biomass, nutrient content, photosynthesis, and transpiration rate and root non-structural carbohydrate levels than younger instars (Womack, 1984; Hower, 1989, Hutchins et al., 1990; Flinn et al., 1990). As leafhopper development proceeds, their energy and nutrient demands increase as does their nutrient procurement abilities. The diminutive mouthparts of first and second instar nymphs may restrict their diets to mesophyll cell contents (Fuentes & Lamp, unpub. data). However, older nymphs and adults are able to probe plant tissues more deeply and exploit phloem sap. Through an unusual (for homopterans) feeding strategy known as lacerate-and-flush, (Miles, 1987; Kabrick & Backus, 1990), multiple phloem cells are rapidly punctured and their contents removed, thereby disrupting translocation and promoting cell collapse (Nielsen et al., 1990; Ecale & Backus, 1995a, b). Subsequent growth of neighboring cells in a saliva-enhanced wound response (Ecale & Backus, 1995b) further disrupts translocation.

In turn, plant life-stage can determine availability of plant tissues for consumption by herbivores. We know little about the workings of this interaction between lacerating insects, like potato leafhopper, and their hosts. We hypothesize that this leafhopper's ability to disrupt phloem translocation or the consequences of disrupted flow may be influenced by the stage of alfalfa stem growth with the harvest regime dictating the developmental cycle of alfalfa stems. During the 10 days following defoliation (i.e., harvest), root starch declines rapidly as carbon is transported upward to regrowing shoots (Smith, 1962). The succulent tissues of young stems may be most vulnerable to attack, and assimilate removal at this time may have the most dire consequences for the current haycrop (Brewer et al., 1986). Root carbohydrate reserves remain low for another 10 days until the bulk of the assimilate stream is diverted toward root tissue as shoots realize their full photosynthetic potential (Pearce et al., 1969; Smith, 1962; Ueno & Smith, 1970). Disruption at this point in the growth cycle may be more difficult for leafhoppers (perhaps, due to greater maturity of tissues and lignification) and may have less severe consequences. However, removal or blockage of assimilate flow to roots can reduce starch accumulation. Taproot starch and nitrogen storage compounds are essential for stand persistence and overwintering success, because plants that do not accumulate root reserves prior to next harvest (some 12–15 days later) suffer reduced rates of regrowth (i.e. may experience larger windows of vulnerability) following subsequent harvests and may even die (Shaw & Wilson, 1986).

How does the rate of photosynthesis respond to the activities of lacerating herbivores? Removing sugars from the assimilate stream may trigger a compensatory increase in photosynthetic rate, but such compensation would also depend on plant developmental stage. Photosynthesis in seedling alfalfa is 'source' limited, suggesting that photosynthetic rate is occurring at or near its maximum (Baysdorfer & Bassham, 1985). However, mature alfalfa plants exhibit 'sink' limited photosynthesis (Baysdorfer & Bassham, 1985; Hodgkinson, 1974). Therefore, predicting the consequences of lacerating herbivore feeding for alfalfa requires knowledge of the developmental stages of alfalfa attacked.

To discover the leafhopper and alfalfa stages that influence phloem translocation and photosynthetic rate, we examined: (1) the extent that phloem disruption by leafhoppers depends on the leafhopper's developmental stage, (2) the extent that phloem disruption by leafhoppers depends on the developmental stage of alfalfa, and (3) the effect of leafhoppers on the photosynthetic rate of alfalfa. Ultimately, this information will permit the design of more realistic, biologically-based, economic injury levels that incorporate the developmental stadia of both organisms. In addition, this information will assist efforts to increase the tolerance of alfalfa to potato leafhopper.

Materials and methods

Disruption of translocation by different leafhopper instars. To test the effects of leafhopper stage on phloem translocation we confined leafhoppers to alfalfa stems, labeled the translocation stream with ^{14}C, and measured the subsequent ^{14}C concentration in four parts of leafhopper-infested and leafhopper-free stems.

Fifteen 10 cm diameter clay pots each containing one susceptible clone of the alfalfa cultivar 'Ranger' were used. Two early vegetative, 10–15 cm tall stems were selected and all remaining stems removed. The uppermost 10 cm of each stem was placed in a clear plexiglas tube 3.8 by 10 cm covered with mesh secured by a perforated tube cap to provide ventilation. A foam plug, cut radially to accept the stem, formed the cage floor. The fourth or fifth fully expanded leaf (below the stem's apex) remained below the foam plug and was designated the source leaf. The petioles of source leaves were covered with two strips of cellophane tape to stabilize them and protect them from injury in handling. Rubber bands attached each cage to a supporting bamboo stake. Thus, leafhoppers had free range of the stem and leaves above the foam plug, but not the source leaf or lower stem.

Potato leafhoppers, obtained from an annually renewed culture reared on fava beans in a greenhouse, were introduced into one cage per plant (i.e. one stem of each plant) via an access hole in the wall of each cage. The other caged stem was the uninjured control. Cages and leafhoppers remained on plants overnight (22 h). Treatments consisted of one of three leafhopper stages: first instar, fourth instar, or adult (selected without regard to sex). We used 6, 4, and 3 individuals of each stage, respectively, in an attempt to ensure plant damage. Thus, there were six treatment combinations, with five replicates of each.

Radiolabeling the translocation stream with ^{14}C took place outside the greenhouse in available natural light. We placed the plants horizontally and sealed two previously designated source leaves, one each from healthy and injured stems, in a 7 by 10 cm plastic bag. We then injected 3 ml of air containing radioactive carbon dioxide ($^{14}CO_2$; 175 μCi) into each bag and sealed the injection hole. Both source leaves were exposed to $^{14}CO_2$ for 30 min. After the fixation period, the bags were removed and the plants were returned to the greenhouse. Translocation of the radiolabel continued for 4 h before processing.

Stems were cut into 3 segments and each segment weighed. The segments consisted of the stem tips (all tissues above the first fully expanded leaf), the upper stem (stem tissue below the tip and above the source leaf), and the lower stem (stem tissue below the source leaf and above the crown). Each stem tip segment was placed immediately in 1 ml of tissue solubilizer (Scintigest, Fisher Scientific, Pittsburgh, PA, USA) in scintillation vials and incubated for 4–5 d at room temperature. The remaining stem segments were stored at $-80\,^{\circ}C$. In preparation for counting, these segments were defrosted, cut into 2–3 mm pieces, and dissolved in 1 ml of tissue solubilizer. After the tissues had dissolved, aqueous scintillation cocktail (Sigma-Fluor, S4273, Sigma Chem. Co., St. Louis, MO, USA) was added to each vial and the ^{14}C radioactivity of the samples was measured in a liquid scintillation counter (Pharmacia LKB, Rockville, MD, USA).

An analysis of variance was performed on the data with the MIXED procedure (SAS, 1997) to test for the fixed effects of leafhopper instar (first, fourth, and adult), injury (no leafhopper injury and leafhopper injury), and stem section (stem tip, upper stem, and lower stem) on the concentration of the labeled assimilate (expressed as log dpm/mg fresh weight). Replicate served as a random effect, and stem section was nested within instar and injury treatment. Heterogeneous variance of residuals was modeled by partitioning the variance into three groups of similar variance. Akaike's information criteria (AIC) was used to ascertain whether the model with partitioned error variance was better than the non-partitioned model (Akaike, 1974). Additionally, to account for correlation among the residuals (plant sections within a stem), various covariance structures were modeled and AIC was used to ascertain which of the covariance structures fit best (Littell et al., 1996). We compared the amount of labeled assimilate in the stem section of injured stems to the same region of healthy stems and made pairwise comparisons for significant differences ($\alpha = 0.05$) using Fisher's Least Significant Difference Test. Only meaningful comparisons were made (i.e., $^{14}CO_2$ concentration within healthy stem section versus the concentration in the matching injured stem section).

Throughout we will use the term exposure to indicate leafhopper access to stems for feeding. For clarity and ease of presentation we will refer to leafhopper exposed stems as injured and leafhopper-free stems as healthy.

Alfalfa growth stage and tolerance to translocation disruption. To test the effects of alfalfa growth stage on disruption of phloem translocation by potato leafhopper, we exposed stems of three growth stages to leafhoppers, labeled the assimilate stream with ^{14}C and measured differences in ^{14}C concentration of four plant parts. We selected five different clones of 'WL320' alfalfa that were grown in 4 by 20 cm plastic tube pots and blocked them by clone. Three different alfalfa growth stages served as treatments:

(1) early vegetative, 10–15 cm; (2) mature vegetative, 18–25 cm; and (3) reproductive, >30 cm with flower buds. Treatment plants were produced by removing and discarding all but the desired size stems. Source leaves were selected and taped as before. Here, the tape also served to prevent leafhopper access to the petioles of source leaves. A tube-cage consisting of two 3.5 by 20 cm clear butyrate tubes joined by a sleeve was placed over each single-stemmed plant. The upper tube was covered by a screen held in place with another tube cap. Screened holes in the sides of the tubes provided ventilation. The lower end of the tube cage fit snugly into the pot and was forced into the soil ca. 1–2 cm. Thus, unlike the previous experiment where leafhoppers only had access to the stem segments above the source leaf, here entire stems and their leaves were exposed to leafhoppers.

A 3 by 2 factorial design (with 3 growth stages and leafhoppers/no leafhoppers as factors) was used to test the tolerance of each growth stage (early vegetative, late vegetative, and reproductive) to leafhopper-induced phloem disruption. There were five replications for each of these six treatment combinations. For each stage, three lab-reared, adult leafhoppers were selected without regard to sex and introduced into each leafhopper treatment cage. The remaining cages did not receive leafhoppers and served as the controls for each growth stage. Plants were arranged randomly by block in a tube-pot rack. Cages and leafhoppers were removed after 20 h.

Radiolabeling the translocation stream with ^{14}C took place outside the greenhouse in full sun. To label the translocation stream, we placed the plants horizontally and sealed two previously designated source leaves – one each from healthy and injured stems – in a 7 by 10 cm plastic bag. We then injected 4 ml of air containing 100 μCi of radioactive carbon dioxide ($^{14}CO_2$) into each bag and exposed the leaves for 15 min. Plants remained outside until the end of the fixation period after which the bags were removed and the plants returned to the greenhouse to translocate the label overnight (18 h). Stems were cut into 5 pieces (tip, upper stem, source leaf, lower stem, and crown) and frozen.

We used a mixed model analysis of variance (Proc Mixed; SAS, 1997) to test for the fixed effects of alfalfa stage (early vegetative, late vegetative, and reproductive), injury (no leafhopper injury and leafhopper injury), and stem section (stem tip, upper stem, lower stem, and crown) on the concentration of the labeled assimilate (expressed as log dpm/mg fresh weight). Clone of 'WL320' alfalfa served as a random effect, and stem section was nested within alfalfa stage and injury treatment. We compared the amount of labeled assimilate in the stem section of injured stems to the same region of healthy stems and made pairwise comparisons for significant differences ($\alpha = 0.05$) using Fisher's Least Significant Difference tests. Only meaningful comparisons were made (i.e., $^{14}CO_2$ concentration within healthy stem section versus the concentration in the matching injured stem section).

Alfalfa growth stage and disruption of photosynthesis by potato leafhopper. To examine the effect of alfalfa growth stage on leafhopper disruption of photosynthesis, we put adult leafhoppers on stems of three growth stages, removed leafhoppers (and cages), and measured photosynthetic rate. Experimental design, plant material, and confinement of leafhoppers were the same as the previous experiment. After removing the leafhoppers and cages in the greenhouse, plants were transferred to full sun (outside the greenhouse) for measurement of photosynthesis (and related processes). We let the plants adjust to full sun for 30 min. prior to measuring photosynthesis. We designated the fourth or fifth fully expanded leaf below the apex for photosynthetic processes measurements. Thus, these leaves were the functional equivalents of the 'source' leaves used in our translocation experiments. We placed each designated leaf in the test chamber of a LI-COR 6200 meter (LI-COR, Inc., Lincoln, NE, USA) and measured its photosynthetic rate in full sun. Therefore, measurements were made on only one leaf per stem with each leaf measurement representing one replicate of each treatment. Moreover, these measurements should indicate the effects of leafhoppers on the photosynthetic rates of the 'source' leaves used above. Afterward, we removed each tested leaf from its stem and measured its area with a Videomex-V (Columbus Instruments, Columbus, OH, USA). Photosynthetic rate was standardized for leaf area, temperature, and humidity in the chamber. We compared the rate of photosynthesis of leaves from injured stems to those from their healthy counterparts. Analysis of variance was conducted to test the effects of growth stage and leafhoppers on the rate of carbon dioxide fixation (μmole CO2 m^{-2} s^{-1}).

Table 1. Analysis of variance table for the effect of leafhopper stage on the translocation of photoassimilate within alfalfa. The source leaf for ^{14}C was between the lower and upper stem. Leafhopper injury was confined to the stem tip and upper stem

Source	Degrees of freedom (NDF, DDF)	F value	P>F
Leafhopper stage (STG)	2, 2.9	3.05	0.19
Leafhopper injury (INJ)	1, 37.3	0.02	0.90
Stem section (SEC)	2, 22.8	8.23	0.002
STG*INJ	2, 40.2	5.08	0.01
STG*SEC	4, 30.9	1.79	0.16
INJ*SEC	2, 22.8	14.60	0.0001
STG*INJ*SEC	4, 30.9	0.63	0.64

Results

Disruption of translocation by different stages of leafhopper. The presence or absence of injury, and the three stages of leafhoppers tested, did not significantly affect the concentration of ^{14}C labeled assimilate recovered among the alfalfa stem sections (Table 1). However, the assimilate did significantly vary among stem sections, with the highest concentration on the lower stem, immediately adjacent to the source leaf. The mean concentration of lower stem, upper stem, and tip was 3.63, 3.24 and 3.07, respectively, expressed as log DPM/mg. The interaction between presence or absence of injury and leafhopper stage was significant, with the log mean assimilate concentration decreasing by 24% when the fourth instar nymph caused the injury, while the log mean concentration increased 18% and 5% when the first instar nymph and the adult stage, respectively, caused the injury. The insignificant three way interaction suggests that the pattern of labeled assimilate concentration across stem sections did not vary by injury from different leafhopper stages.

The LSD tests demonstrated that the fourth instar nymphs had a greater effect than either adults or first instar nymphs on disruption of the upward flow of assimilates to the stem tip and upper stem (Table 2). A similar, but smaller trend was also found for adults. However, this difference was not significant, possibly because of the confounding variable of adult gender. We did not determine gender of the adults used in the experiment. Yet, female potato leafhoppers cause more injury than males (Hower, 1989), and males are more active and spend less time feeding in cages than females (Lamp, pers. obs.). First instar nymphs were unable to diminish the flow of assimilates to stem tips when applied at a density of six per stem. In contrast to the disruption of the upward flow of assimilates, the first instar nymphs and adults caused a significant increase in the concentration of assimilates in the lower stem tissue, while the fourth instar nymphs did not.

No significant differences were found in ^{14}C labeled assimilate recovered from source leaves of healthy versus injured plants (prob. F>0.10).

Alfalfa growth stage and translocation disruption. The extent that leafhoppers diminished the flow of labeled assimilates depended on the developmental stage of alfalfa as shown by the significant interaction between alfalfa stage and leafhopper injury (Table 3). The three-way interaction was also significant, indicating that the pattern of labeled assimilates across stem sections did vary depending on alfalfa stage of development. However, few significant comparisons between the amount of ^{14}C translocated to the same healthy versus injured stem parts (i.e. within each part category: stem tips, upper stem, lower stem and crown) were found (Table 4). Stems exposed to adult leafhoppers during the three developmental stages tested contained altered concentrations of labeled assimilate in their tips. Eighty-nine times less radioactivity was recovered from stem tip tissues of early vegetative stems exposed to leafhoppers compared to healthy stem tips. The tips of mature (late) vegetative (18–25 cm) stems exhibited a similar trend, containing twelve times less ^{14}C than their healthy counterparts. Moreover, leafhoppers reduced the levels of labeled assimilate recovered from the tips of early vegetative and late vegetative stems to a significantly greater extent than reproductive stems. The amount of label recovered from crown tissues was low, near the limit of detection, however there was significantly less labeled assimilate in crown tissues of injured plants compared to crown tissues of healthy plants.

As before, no differences between source leaves from healthy or injured plants were observed (alfalfa stage df=2, F=2.34, prob.>F = 0.12; leafhopper presence df=1, F=0.19, prob.>F = 0.67; stage X presence interaction df=2, F=1.75, prob.>F = 0.20).

Alfalfa growth stage and disruption of photosynthesis by potato leafhopper. Adult leafhoppers had no effect on net photosynthesis, stomatal resistance, or stomatal conductance (photosynthesis: alfalfa stage

Table 2. Means and Least Significant Difference Tests within rows for the effect of leafhopper stage on the translocation of photoassimilate within alfalfa. The distribution of photoassimilates is expressed as mean concentration (and standard error) of radiolabelled ^{14}C

Leafhopper stage	Stem section	Recovered ^{14}C photoassimilate (log DPM/mg)		Least-squares difference test (P>t)
		Healthy	Injured	
First instar	Stem tip	3.51 (0.33)	3.66 (0.36)	0.73
	Upper stem	3.26 (0.39)	3.73 (0.35)	0.28
	Lower stem	3.30 (0.29)	4.45 (0.12)	0.01
Fourth instar	Stem tip	3.57 (0.30)	2.04 (0.13)	0.0008
	Upper stem	3.47 (0.22)	2.41 (0.57)	0.02
	Lower stem	3.40 (0.25)	3.51 (0.56)	0.80
Adult	Stem tip	3.15 (0.25)	2.51 (0.41)	0.14
	Upper stem	3.21 (0.33)	3.34 (0.30)	0.75
	Lower stem	3.03 (0.23)	4.06 (0.07)	0.02

Table 3. Analysis of variance table for the effect of alfalfa developmental stage and leafhopper injury on the translocation of photoassimilate within alfalfa. The source leaf for ^{14}C was between the lower and upper stem. The entire stem was exposed to leafhoppers

Source	Degrees of freedom (NDF, DDF)	F value	P>F
Alfalfa stage (ALF)	2, 52.9	4.51	0.016
Leafhopper injury (INJ)	1, 52.9	5.66	0.021
Stem section (SEC)	3, 61	193.32	0.0001
ALF*INJ	6, 61.1	4.10	0.022
ALF*SEC	6, 61.1	2.24	0.052
INJ*SEC	3, 61	7.89	0.0002
ALF*INJ*SEC	6, 61.1	2.50	0.032

df=2, F=0.67, prob.>F = 0.52; leafhopper presence df=1, F=1.09, prob.>F =0.31) of the leaves examined. No evidence for photosynthetic compensation was found. Only the analysis of variance for stomatal conductance before leafhopper exposure had significant effects (stomatal conductance increased with stem growth stage). Therefore, carbon removal or phloem blockage by leafhoppers did not significantly alter photosynthetic rate of the fourth (or fifth) fully expanded leaf below the apex of these stems.

Discussion

The transport of photoassimilate links photosynthetic regions to regenerative and storage organs of plants. Thus, disruption of the translocation of photoassimilates can have profound effects on longevity and growth processes in plants. The injury to alfalfa stems by potato leafhopper seems to fit this paradigm. We found that feeding by the leafhopper disrupts translocation, but at least part of this effect depends upon the developmental stage of both host and insect. The insect's capacity to disrupt phloem translocation was a function of its developmental stage. For example, fourth instar nymphs were more injurious to the upward flow of photoassimilates than first instar nymphs of the leafhopper. Moreover, early vegetative stages of the host were most vulnerable to the disruption of the upward assimilate stream by adults. Therefore, the timing of insect attack and the developmental stages of plant and insect at the time of attack are intimately related to the level of injury imposed.

We found no alteration of the rate of photosynthesis (and related processes) for leaves of equivalent location and treatment to the source leaves used here and in our other translocation studies (Nielsen et al., 1990). This indicates that adult leafhoppers have no discernable effects on mature, fully-expanded leaves located five or six nodes below the apex of the stem. Furthermore, there were no differences in the fixation of labeled carbon by the source leaves. Therefore, differences in the amount of ^{14}C in particular plant

Table 4. Effect of leafhopper injury on the translocation of photoassimilate within alfalfa of three developmental stages. The distribution of photoassimilates is expressed as mean concentration (and standard error) of radiolabelled ^{14}C. The source leaf for ^{14}C was between the lower and upper stem. The entire stem was exposed to leafhoppers

Alfalfa development stage	Stem section	Recovered ^{14}C photoassimilate (log DPM/mg)		Least-squares difference test (P>t)
		Healthy	Injured	
Early vegetative	Stem tip	4.03 (0.14)	2.08 (0.35)	0.0001
	Upper stem	3.74 (0.18)	3.87 (0.26)	0.75
	Lower stem	3.35 (0.28)	2.94 (0.11)	0.21
	Crown	1.07 (0.35)	0.09 (0.44)	0.02
Late vegetative	Stem tip	3.67 (0.22)	3.24 (0.21)	0.37
	Upper stem	3.57 (0.16)	3.73 (0.18)	0.63
	Lower stem	2.90 (0.22)	2.92 (0.16)	0.95
	Crown	0.43 (0.29)	−0.44 (0.11)	0.01
Reproductive	Stem tip	2.89 (0.36)	3.20 (0.37)	0.45
	Upper stem	3.12 (0.31)	3.26 (0.30)	0.73
	Lower stem	2.12 (0.30)	2.58 (0.27)	0.28
	Crown	0.30 (0.23)	−0.15 (0.44)	0.40

parts (e.g., tips) probably cannot be ascribed to differences in carbon fixation by leaves from healthy versus injured plants, but most likely are attributable to translocation disruption by potato leafhopper. The differences others have found in photosynthesis may be due to injury that is more severe and longer in duration (Womack, 1984; Hutchins et al., 1990; Flinn et al., 1990). Moreover, these studies examined whole plant rates of photosynthesis, whereas we measured photosynthesis in individual leaves and, in particular, only leaves at this distance below the apex.

In the first experiment, the ability of different leafhopper stages to disrupt phloem translocation depended upon their size and age. The fourth instar nymph treatment significantly reduced the upward flow of photoassimilates, while the first instar nymphs and adults did not. This result contrasts with the impact of adults during the second experiment (see discussion below). Other researchers have found similar effects of leafhopper nymph instar on alfalfa yield and quality, photosynthesis, transpiration rate, and root non-structural carbohydrate levels in the field (Womack, 1984; Hutchins et al., 1990; Flinn et al., 1990). Why the first instar nymphs were unable to disrupt translocation is unclear. Perhaps, alfalfa stems were too tough for nymphal stylets or its phloem tissue was beyond the reach of their mouthparts. Brewer et al. (1986) found potato leafhopper resistance of alfalfa correlated with stem hardness and glandular hairs. When feeding on alfalfa, first instar nymphs have been observed primarily on leaf tissue and progress to stem tissue in later instars (i.e. third-fifth stadia; Fuentes & Lamp, *unpubl.*). Our results demonstrated that even greater numbers of first instar nymphs (twice as many as adults) were unable to disrupt upward translocation.

Although the first instar nymphs and adults did not significantly affect upward translocation, these stages were associated with significantly greater photoassimilate concentrations in the lower stem section compared to the healthy control plants. This may have been the result of redirection of labeled photoassimilates away from the injured regions of the stem, since leafhoppers were confined to the upper stem and tip regions. The difference between these two stages and the fourth instar nymph could be the result of feeding site preferences. Although we did not quantify the exact feeding sites in these tests, we have observed that early instars and adults sometimes feed at the lowest available sites on caged stems, and perhaps feeding site will influence the direction of photoassimilates. Further testing, specifically by confinement of specific stages onto specific tissues, is needed to test this hypothesis. As the alfalfa plant develops, leafhopper induced disruption of translocation (i.e., significant changes in the rate or amount of photoassimilate transport to growing points of stems) and consequent stem

injury should occur primarily in vegetative stages prior to the redirection of photoassimilate to taproots and crowns. After defoliation (i.e. harvest), carbohydrates and nitrogenous compounds are mobilized to support nutrient transport from the root to developing buds and stems (Smith, 1962; Ueno & Smith, 1970; Volenec et al., 1996). This decline in root nonstructural carbohydrates continues for about 15 days after which carbohydrates re-accumulate, achieving maximum levels near the end of the 32–35 day growth cycle (Pearce et al., 1969; Ueno & Smith, 1970; Rapoport & Travis, 1984). Such sinks (e.g., crown and buds) determine the rate and direction of translocation (Baysdorfer & Bassham, 1985). As expected in our second experiment, we observed that injury is associated with reduced upward translocation in early vegetative stems (i.e., well before the end of the growth cycle and reversal of the predominant direction of assimilate flow). However, we also observed that injury is associated with reduced rootward translocation of both early and late vegetative plants as determined by concentrations of label in crown tissues. This observation may be premature, because of the small amount of labeled assimilate recovered from crowns.

Because of differences in experimental protocol (e.g., caging treatments, tissues sampled), direct comparison of the two translocation experiments is difficult. However, we can compare the results of both experiments for the disruption by adults of upward photoassimilate flow by comparing the concentration of label in stem tips of injured versus healthy plants in the early vegetative stage. Although the trend for means for healthy and injured plants were similar in both experiments (4.03 log DPM/mg versus 2.08, respectively, for the second experiment, and 3.15 versus 2.51, respectively, for the first experiment), the difference was significant for the second experiment (Table 4) but not significant for the first experiment (Table 2). Since we did not determine the gender of the adults used in either experiment, a gender effect may have contributed to the contrasting results of the two experiments. As mentioned earlier, female potato leafhoppers cause more injury than males (Hower, 1989), and males are more active and spend less time feeding in cages than females (Lamp, pers. obs.). Thus, the lack of knowledge of the gender and the ability of the adults to spend more time off the stem than nymphs may explain the difference.

Our results on leafhopper disruption of translocation suggest that current economic thresholds need revision. Reduced apical translocation associated with feeding injury is expected to immediately impair leaf development and stem elongation, and the level of this impact is greatest during early vegetative stages and becomes less as the plant develops. Thus, our results corroborate the use of stem height as a measure of susceptibility in current decision-making guidelines (Cuperus et al., 1983; Hellman et al., 1995). However, the current guidelines do not distinguish between nymphs and adults. According to published guidelines, a sample consisting of both adults and nymphs is collected by sweep net and the total number of leafhoppers (all stages combined) is compared to the economic threshold (Hellman et al., 1995). Yet, the injury caused by nymphs and adults differs in intensity and, possibly, the type of physiological disruption. In addition, present guidelines do not consider long term effects of feeding on subsequent harvests or plant persistence, yet disruption of translocation may affect carbohydrate storage in root and crown tissue. Therefore, more data are needed to further revise current guidelines.

A clearer picture of the extent and rapidity of injury produced by potato leafhopper is emerging, aided by our current understanding of leafhopper feeding behavior and its impact on cellular structure (Ecale & Backus, 1995a, b). For alfalfa, disruption of one physiological process, phloem translocation, depends on the developmental stage of both host and insect. Further elucidation of this interaction will enhance efforts to manage this key alfalfa pest and contribute to our understanding of plant-insect interactions, particularly, those between sap-feeding herbivores and their hosts.

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